

Methylamp™ Global DNA Methylation Quantification Ultra Kit

Base Catalog # P-1014B

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The Methylamp™ Global DNA Methylation Quantification Ultra Kit is suitable for detecting global DNA methylation status using genomic DNA isolated from any species such as mammals, plants, fungi, bacteria, and virus in a variety of forms including cultured cells, fresh and frozen tissues, paraffin-embedded tissue, plasma/serum sample, body fluid sample, etc.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

KIT CONTENTS

Component	48 Assays Cat. #P-1014B-48	96 Assays Cat. #P-1014B-96	Storage Upon Receipt
GU1 (10X Wash Buffer)	15 ml	30 ml	4°C
GU2 (DNA Binding Solution)	1.5 ml	3 ml	RT
GU3 (Positive Control, 100 µg/ml)*	10 µl	20 µl	-20°C
GU4 (Block Solution)	10 ml	20 ml	4°C
GU5 (Capture Antibody, 1000 µg/ml)*	5 µl	8 µl	4°C
GU6 (Detection Antibody, 400 µg/ml)*	10 µl	20 µl	-20°C
GU7 (Enhancer Solution)*	10 µl	20 µl	-20°C
GU8 (Developer Solution)	5 ml	10 ml	4°C
GU9 (Stop Solution)	3 ml	6 ml	RT
Negative Control DNA (50 ng/µl)*	10 µl	20 µl	-20°C
8-Well Assay Strips (With Frame)	6	12	4°C
User Guide	1	1	RT

* For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **GU3**, **GU6**, **GU7**, and **Negative Control DNA** at -20°C away from light; (2) Store **GU1**, **GU4**, **GU5**, **GU8**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature.

Note: Check if wash buffer, **GU1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

The kit is stable for up to 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microplate reader with the ability to read at 450 nm
- Pipette and pipette tips
- 1.5 ml microcentrifuge tubes

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of Methylamp™ Global DNA Methylation Quantification Ultra Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Usage Limitation: The Methylamp™ Global DNA Methylation Quantification Ultra Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The Methylamp™ Global DNA Methylation Quantification Ultra Kit and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

Epigenetic alterations of genomic DNA play a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Region-specific DNA methylation is mainly found in 5'-CpG-3' dinucleotides within the promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells. Global DNA hypomethylation is likely caused by methyl-deficiency due to a variety of environmental influences, and has been proposed as a molecular marker in multiple biological processes such as cancer. It is well demonstrated that the decrease in global DNA methylation is one of the most important characteristics of cancer. Thus, the quantification of global methylation in cancer cells could provide very useful information for detection and analysis of this disease.

There are several methods to measure global DNA methylation levels, including mass spectrometry, enzymatic degradation and analysis, and immunohistochemical staining. However, such methods have many disadvantages including the need for special equipment, long protocols, low sensitivity, and/or radioactivity. To address these problems, Epigentek developed an ELISA based colorimetric kit, Methylamp™ Global DNA Methylation Quantification Ultra Kit. This kit is faster, more accurate, and much more convenient than conventional methods.

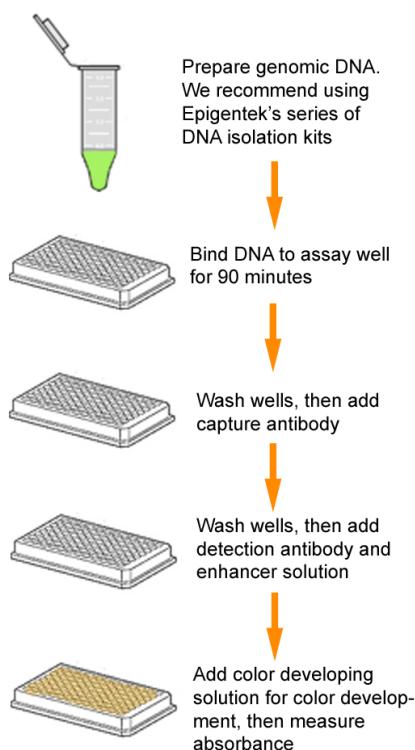
The Methylamp™ Global DNA Methylation Quantification Ultra Kit has the following advantages and features:

- Colorimetric quantification without radioactivity, extraction, and chromatography.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Fast procedure, which can be finished in less than 4 hours.
- High sensitivity – detection limitation can be as low as 0.2 ng of methylated DNA.
- Universal positive control suitable for quantifying methylated DNA from any species.



PRINCIPLE & PROCEDURE

The Methylamp™ Global DNA Methylation Quantification Ultra Kit contains all the reagents necessary for a quantification of global DNA methylation. In this assay, DNA is immobilized to strip wells specifically coated with DNA affinity substance. The methylated fraction of DNA can be recognized by 5-methylcytosine antibody and quantified through an ELISA-like reaction. The amount of methylated DNA is proportional to the OD intensity.



Schematic procedure of the Methylamp™ Global DNA Methylation Quantification Kit

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

1.

Prepare DNA by using your own successful method. For your convenience and the best results, Epigentek offers a series of DNA isolation kits which is optimized for extracting DNA from cultured cells, tissues, body fluids, and paraffin sections.

2.

- Predetermine the number of strip wells required for your experiment. Remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).
- Predetermine your plate arrangement for the sample wells, positive control wells, and negative control wells.

c. Dilute **GU1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g., 1 ml of **GU1** + 9 ml of distilled water).

3.

a. Sample: Add 28 μ l of **GU2** solution into the sample well followed by adding 2 μ l (100-200 ng) of sample DNA.

b. Positive Control – Single Point: Dilute **GU3** with **GU2** at a 1:20 ratio (e.g., 1 μ l of **GU3** + 19 μ l of **GU2**). Add 28 μ l of **GU2** into the positive control well, followed by adding 2 μ l of the **Diluted GU3** solution (10 ng/well); or

Positive Control – Standard Curve: Dilute **GU3** to 0.2-10 ng/ μ l, respectively with **GU2**. Add 28 μ l of **GU2** into the positive control well, followed by adding 2 μ l of each **Diluted GU3** solution to generate a standard curve (make 4 to 6 points, such as 0.4, 1, 2, 5, 10, and 20 ng/well).

c. Negative Control*: Add 28 μ l of **GU2**, followed by adding 2 μ l of **Negative Control DNA** into the negative control wells.

**Required for background; cannot be substituted with DNA-free control.*

d. Shake the plate frame to allow the solution to cover the entire surface of each well's bottom.

e. Incubate the wells at 37°C (with no humidity) for 40 min, followed by incubation at 60°C (with no humidity) for 35 to 40 min to evaporate the solution and dry the wells.

Note: The non-evaporated solution might accumulate along the edges at the bottom of the well. Make sure the well is completely dry by slightly tilting the well and aspirating against the edge with a P-10 or P-20 pipette. If there is still residue solution, extend incubation time for an additional 5-10 min at 60°C to dry the well. It is normal to see white salt precipitates in the wells, which will eventually be washed out in Step 5.

4.

Add 150 μ l of **GU4** to each dried well. Incubate at 37°C for 30 min.

5.

Aspirate and wash each well with 150 μ l of the **Diluted GU1** each time for three times.

6.

Dilute **GU5** (at a 1:1000 ratio) to 1 μ g/ml with the **Diluted GU1**. Add 50 μ l of **Diluted GU5** to each well and incubate at room temperature for 60 min.

7.

Aspirate and wash each well with 150 μ l of the **Diluted GU1** each time for four times.

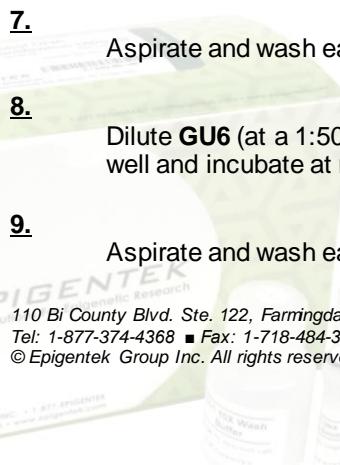
8.

Dilute **GU6** (at a 1:5000 to 1:10,000 ratio) with the **Diluted GU1**. Add 50 μ l of **Diluted GU6** to each well and incubate at room temperature for 30 min.

9.

Aspirate and wash each well with 150 μ l of the **Diluted GU1** each time for five times.

10.

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10.

Dilute **GU7** (at a 1:5000 to 1:10,000 ratio) with the **Diluted GU1**. Add 50 μ l of **Diluted GU7** to each well and incubate at room temperature for 30 min.

11.

Aspirate and wash each well with 150 μ l of the **Diluted GU1** each time for five times.

12.

Add 100 μ l of **GU8** to each well and incubate at room temperature for 1-5 min away from light. Begin monitoring color development in the sample and the control well for a medium blue color.

13.

Add 50 μ l of **GU9** to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color will change to yellow and the absorbance should be read on a microplate reader at 450 nm within 2 to 15 min.

For simple calculation of DNA methylation, use the following formula (the amount of the positive control is 10 ng and sample DNA is 100 ng):

$$\text{Methylation \%} = \frac{(\text{Sample OD} - \text{Negative Control OD})/X^*}{(\text{Positive Control OD} - \text{Negative Control OD}) \times 10} \times 100\%$$

For accurate calculation of DNA methylation, plot OD value versus amount of **GU3** and determine the slope as OD/ng. Then calculate the amount of methylated DNA using the following formulas:

$$\text{Methylated DNA (ng)} = \frac{\text{Sample OD} - \text{Negative Control}}{\text{Slope}}$$

$$\text{Methylation \%} = \frac{\text{Methylated DNA Amount} / X^*}{\text{Sample DNA Amount Added}} \times 100\%$$

* X is the GC content of the species DNA (e.g., GC content is 41% human genomic DNA, 42% for mouse and rat, 35% for *A thaliana*, 38% for yeast, etc.)

TROUBLESHOOTING

Problem	Possible Causes	Suggestions
No signal in both the positive control and the sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order and if some steps in the protocol may have been omitted by mistake.
	The well is not completely dried.	Ensure the well is incubated with no humidity and dry the well before adding

		block buffer.
	The well is incorrectly washed before DNA coating.	Ensure the well is not washed prior to adding the control DNA.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
No signal or weak signal in only the positive control wells	The positive control DNA is insufficiently added to the well in Step 3b.	Mix the control DNA thoroughly before adding to the wells. Check for proper dilution ratios. Ensure sufficient amount of control DNA is added.
	The GU3 Positive Control is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this user guide or storage of GU3 Positive Control.
High background present in the negative control wells.	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by sample or positive control DNA.	Ensure the well is not contaminated from adding sample or positive control DNA accidentally or from using contaminated tips.
	Insufficient or no blocking.	Ensure the well is properly blocked with GU4 Block Solution in Step 4a.
	Over-development.	Decrease the development time in Step 11 before adding GU9 Stop Solution in Step 12.

RELATED PRODUCTS

DNA Sample Preparation

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1007	FitAmp™ Gel DNA Isolation Kit
P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit

Global DNA Methylation Quantification

P-1021	SuperSense™ Methylated DNA Quantification Kit
P-1036	MethylFlash™ Hydroxymethylated DNA Quantification Kit

